EXHIBIT I

CHEMICAL CARCINOGENESIS STUDIES IN NONHUMAN PRIMATES

R. H. Adamson and S. M. Sieber

Laboratory of Chemical Pharmacology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205

INTRODUCTION

A wide variety of chemicals has been shown to be capable of inducing malignant tumors in rodents. However, rodent data are difficult to extrapolate to man, and the problem of risk assessment for humans remains largely unsolved. Extrapolation of rodent carcinogenesis data to man is particularly difficult because of known species differences in metabolic pathways (both activation and detoxification), involving potentially carcinogenic chemicals. Nonhuman primates are phylogenetically closer to man than rodents, and many metabolic pathways in monkeys parallel those in humans (1,2). For this reason, nonhuman primates may provide a more suitable model for the study of potential carcinogens, particularly those requiring metabolic activation and those detoxified by various enzyme systems.

Chemical carcinogenesis studies in nonhuman primates have been conducted for the past 20 years in this laboratory. During this time a considerable amount of information on the spontaneous tumor incidence in a closed colony of nonhuman primates, primarily Old World monkeys, has been amassed. In addition, the carcinogenicity of chemicals and other substances has been assessed. The results of these ongoing studies are described below.

METHODS

Animals

The present colony, consisting of 545 animals, is comprised of four species: Macaca mulatta (rhesus), Macaca fascicularis (cynomolgus), Cercopithecus aethiops (African green), and Galago crassicaudatus (bush babies). Fifty-four of these monkeys are adult breeders that supply the newborns for experimental studies. The original goal of this program was to establish whether selected carcinogens would have any effect in primates of any species, and as wide a range of species as was practical was deliberately chosen. African green (Gr), cynomolgus (Cy), rhesus (Rh), and occasionally (Cy x Rh)F_1 hybrids were generally included in each protocol, and were assigned randomly as they became available. Accordingly, different numbers of each species were used from one study to the next. The majority of the animals are housed in an isolated facility that contains only animals committed to this study, and with the exception of the breeding colony, most animals are housed in individual cages. Details of maintenance and management procedures and the method used to rear neonates have been described elsewhere (3). Newborns produced by the breeding colony are taken within 12 hours of birth to a nursery staffed on a 24-hour basis. The administration of test compounds is usually initiated within 24 hours of birth and continues until a tumor is diagnosed or until a predetermined exposure period has been completed.

A variety of clinical, biochemical, and hematological parameters is monitored weekly or monthly, not only to evaluate the general health status of each animal, but also for the early detection of tumors. Surgical procedures are performed under phencyclidine hydrochloride, Ketamine, or sodium pentobarbital anesthesia. All animals that die or are sacrificed are carefully necropsied and the tissues subjected to histopathologic examination.

Compounds Under Evaluation

A wide variety of substances has been or is being evaluated (Table 1). The compounds are administered subcutaneously (sc), intravenously (iv), intraperitoneally (ip), or orally (po). For po administration to newborn monkeys, the compound is added to Similac formula at feeding time; when the monkeys are six months old, carcinogens given po are generally incorporated into a vitamin mixture given to the monkeys as a vitamin sandwich on a half slice of bread. The dose level chosen is dependent on the chemical under evaluation. The therapeutic agents under test are administered at doses likely to be encountered in a clinical situation. Other

Table 1. Substances Tested for Carcinogenic Activity in Nonhuman Primates.

Common Name	Alternate or Chemical Name(s)
Therapeutic Agents	
Procarbazine	N-Isopropyl-α-(2-methylhydrazino)-p-toluamide
Adriamycin	Daunorubicin
Melphalan	L-p-Bis(2-chloroethyl)aminophenyl-alanine; phenylalanine mustard
Azathioprine	6-[(1-Methyl-4-nitroimidazol-5-yl-thio]purine; Imuran
Cyclophosphamide	2-[Bis(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine-2-oxide; Cytoxan
Food Additives and Environmental Contaminants	
Aflatoxin B_1	--
MAM acetate[a]	Methylazoxymethanol acetate
Sterigmatocystin	
Cyclamate	Cyclohexane sulfamic acid, sodium salt
Saccharin	2,3-Dihydro-3-oxobenzisosulfonazole; 2-sulfobenzoic acid imide
DDT	1,1,1-Trichloro-2,2-bis(p-chlorophenyl)ethane
Arsenic	Sodium arsenate
Cigarette smoke condensate	--
Model Rodent Carcinogens	
Urethane	Ethyl carbamate
3-Methylcholanthrene	--
Dibenzo[a,i]pyrene	
Dibenz[a,h]anthracene	
2-Acetylaminofluorene	
2,7-Bis(acetylamino)fluorene	
4-Dimethylaminoazobenzene	Butter yellow
3'-Methyl-4-dimethylaminoazobenzene	--
N-Nitroso Compounds	
Nitrosomethylurea	1-Methylnitrosourea
1-Nitroso-1-methyl-3-nitroguanidine	N-Methyl-N'-nitro-N-nitrosoguanidine
Nitrosopiperidine	1-Nitrosopiperidine
Nitrosodimethylamine	Dimethylnitrosamine
Nitrosodiethylamine	Diethylnitrosamine
Nitrosodi-n-propylamine	Dipropylnitrosamine

[a]Some animals also received cycasin, methyl azoxymethanol-β-D-glucopyranoside, or crude cycad meal containing cycasin.

substances, such as environmental contaminants and food additives, are usually given at levels 10 to 40 times higher than the estimated human exposure level. The remainder of the chemicals tested are administered at maximally tolerated doses that, on the basis of weight gain, blood chemistry, hematology findings, and clinical observations, appear to be devoid of acute toxicity.

RESULTS

Therapeutic Agents

Of the cancer chemotherapeutic agents under test, only procarbazine (4) and adriamycin (5) have thus far provided evidence of being carcinogens in nonhuman primates. Table 2 compares the tumor incidence in a group of 50 monkeys receiving long-term procarbazine treatment with that in a control population of 219. The group of 50 monkeys received procarbazine by ip, sc, and/or po routes, and 41 have been necropsied. Thirteen of the necropsied monkeys were diagnosed with tumor, yielding an overall tumor incidence of 26%. In contrast, 7 out of 219 (3.2%) control monkeys have developed spontaneous tumors over the past 20 years. Controls consisted of 45 Gr, 91 Rh, and 83 Cy monkeys; among these, tumors have developed in 3 Gr, 3 Rh, and 1 Cy. Approximately half of the procarbazine-induced neoplasms were acute leukemia, chiefly myelogenous (see Table 3), that arose in monkeys after latent periods ranging between 16 and 143 months (average 77 months). The monkeys developing leukemia had ingested an average cumulative procarbazine dose of 45.53 g (range 2.69 to 103.68 g). Table 4 lists the six solid tumors induced by procarbazine; half of these tumors were osteosarcomas, two were hemangiosarcomas, and one was a lymphocytic lymphoma. The solid tumors arose in monkeys ingesting an average cumulative procarbazine dose of 56.88 g (range 23.85 to 154.37 g) and were diagnosed after an average latent period of 98 months (range 68 to 148 months).

An evaluation of the carcinogenic potential of adriamycin was initiated approximately 6 years ago in a group of 10 monkeys. The original plan was to administer 30 doses of adriamycin at 1 mg/kg (30 mg/kg); when this total dose was attained, the monkeys were to be observed for the remainder of their lives. However, a monkey died with acute clinical signs of congestive heart failure (ascites, periorbital edema, and rapid and labored respiration) after receiving 26 doses of adriamycin (26 mg/kg). Dosing of all monkeys in this group was terminated approximately one month later, but in the following two months, five more monkeys developed congestive heart failure and died, and two additional monkeys died three and four months later, respectively. Necropsy and histopathologic examination of tissue from all eight animals revealed lesions typical of adriamycin cardiomyopathy. These

Table 2. Summary of Control and Procarbazine-Treated Monkeys[a]

Group	No. Alive	No. Dead			No. of Monkeys
		Without Tumor	With Tumor	(%)	
Procarbazine	9	28	13	(26)	50[b]
Control[c]	129	83	7	(3.2)	219

[a]From 1961 to 1981.
[b]23 Rh, 16 Cy, and 11 Gr.
[c]Includes non-treated animals, breeders, and vehicle-treated controls.

monkeys had received an average cumulative adriamycin dose of 74.4 mg divided among 23 to 27 monthly doses (see Table 5). Another monkey was sacrificed because of marked weight loss and anorexia of approximately five weeks duration. Although no specific abnormalities were noted at necropsy of this monkey except for enlarged mesenteric lymph nodes, histological examination of sections of bone marrow yielded a diagnosis of acute myeloblastic leukemia. This animal had received a cumulative adriamycin dose of 77.8 mg, and the leukemia was diagnosed two months after the last dose of adriamycin. The tenth monkey in this series is alive and without evidence of illness; it received 25 injections of adriamycin (72 mg), the last administered 42 months ago.

We are currently repeating this study, using 2 groups of 10 monkeys each; the monkeys are receiving monthly intravenous injections of adriamycin at 0.2 and 0.4 mg/kg, and dosing will be terminated when the monkeys have received a cumulative adriamycin dose of 60 mg. To date, the monkeys receiving adriamycin at 0.2 and 0.4 mg/kg have been given cumulative drug doses of 15.6 and 21.8 mg, respectively. None of the monkeys has developed signs of congestive heart failure or other indications of ill health.

The remainder of the chemotherapeutic agents under test (melphalan, azathioprine, and cyclophosphamide) have not as yet provided any indication of carcinogenicity. Twenty monkeys are receiving melphalan po at a dose of 0.1 mg/kg, 5 days/week. The first group of 10 monkeys was put on test 64 months ago, and since that time has ingested an average cumulative melphalan dose of 128 mg/kg. The second group of 10 monkeys was put on test 10 months later, and the average cumulative melphalan dose ingested

Table 3. Acute Leukemias Induced in Monkeys by Procarbazine.

Monkey No.	Species[a]	Sex	Dose (mg/kg)	Route[b]	Months Dosed	Total Dose (gm)	Latent Period (mo)[c]	Type of Leukemia
267D	Rh	F	50 10	sc po	15 1	2.69	16	Myelogenous
733I	Cy	M	20	ip	57	7.29	57	Undifferentiated
726I	Cy	M	20	ip	68	16.24	68	Myelogenous
313E	Rh	F	5-50 10	sc po	35 33	37.25	68	Myelogenous
567G	Rh	F	10-25 10	sc po	6 71	49.99	77	Myelogenous
13T	Rh	M	25-50 10	sc po	36 73	101.64	109	Myelogenous
336E	Rh	F	10-50 10	sc po	33 110	103.68	143	Myelogenous
Average						45.53	77	

[a]Rh = rhesus; Cy = cynomolgus.
[b]Doses for sc and ip given 1 day/week; po doses given 5 days/week.
[c]Latent period is the number of months from first dose to diagnosis of leukemia.

Table 4. Solid Tumors Induced in Monkeys by Procarbazine.

Monkey No.	Species[a]	Sex	Dose (mg/kg)	Route[b]	Months Dosed	Total Dose (gm)	Latent Period (mo)[c]	Tumor(s) Diagnosed
734I	Rh	M	10-20 10	ip po	62 5	23.85	71	Hemangioendothelial sarcoma; kidney
731I	Rh	F	10-20 10	ip po	63 5	24.22	68	Osteosarcoma; humerus
314E	Cy	F	5-50 10	sc po	35 62	32.88	97	Hemangiosarcoma; spleen
315E	Cy	M	5-50 10	sc po	35 63	50.18	98	Lymphocytic lymphoma
333E	Cy	F	10-50 10	sc po	33 70	57.05	103	Osteosarcoma; jaw
557G	Cy	F	10-50 10	sc po	7 140	154.37	148	Osteosarcoma; humerus
Average						56.88	98	

[a]Rh = rhesus; Cy = cynomolgus.
[b]Doses for sc and ip given 1 day/week; po doses given 5 days/week.
[c]Latent period is the number of months from first dose to diagnosis of leukemia.

Table 5. Effects of Adriamycin in Monkeys[a]

No. of Monkeys	No. of Doses[b]	Avg. Total Dose (mg)	Cause of Death
8[c]	23-27	74.4[d]	Congestive heart failure
1 (Rh)	27	77.8	Acute myeloblastic leukemia (Rh)
1 (Rh)	25	72.0	None

[a]Summary of first study.
[b]Monkeys were given monthly iv doses of adriamycin (1 mg/kg) beginning at two months of age.
[c]4 Cy, 4 Rh.
[d]Range is 66.2 to 80.6.

by this group of monkeys is 108 mg/kg. None of the monkeys has died, and none has demonstrated any clinical signs of ill health.

Two groups of monkeys are being given azathioprine po, 5 days/week. A group of 14 monkeys has been receiving the compound at 2.0 mg/kg for an average of 38 months (range 9 to 43 months). During this period the monkeys have received a cumulative azathioprine dose averaging 1.52 g/kg (range 0.36-1.72 g/kg). A second group of 10 monkeys began receiving azathioprine (5.0 mg/kg) an average of 28 months ago (range 25 to 29 months); these monkeys have ingested an average of 2.8 g/kg azathioprine (range 2.5-2.9 g/kg). All monkeys receiving azathioprine appear healthy and are without clinical signs of ill health.

A study of the potential carcinogenicity of cyclophosphamide was recently initiated. A group of 20 monkeys is receiving cyclophosphamide po 5 days/week. Dosing (3 mg/kg) begins when the monkeys are six months old, and the dose is increased to 6 mg/kg when the monkeys are one year old. The study has been under way for an average of only six months; during this period an average cumulative cyclophosphamide dose of 0.78 g (range 0.04 to 2.25 g) has been administered. Thus far, none of the monkeys has died, and there is no evidence of toxicity on this dosage schedule.

Food Additives and Environmental Contaminants

Despite prolonged treatment with most of the food additives and environmental contaminants listed in Table 1, only aflatoxin B_1 (AFB_1) and methylazoxymethanol-acetate (MAM-acetate) have induced

tumors in monkeys. AFB_1 has been under evaluation for the past 15 years (6). A total of 45 monkeys, chiefly rhesus and cynomolgus, has received AFB_1 ip (0.125 to 0.25 mg/kg) and/or po (0.1 to 0.8 mg/kg) for two months or longer, and six are currently alive and without evidence of tumor. Eighteen of the 39 monkeys necropsied to date developed one or more malignant neoplasms, yielding an overall tumor incidence of 40% (see Table 6). A summary of the tumors diagnosed in these monkeys is shown in Table 7. Seven of the 18 tumor-bearing monkeys developed hemangioendothelial sarcomas of the liver, five had bile duct or gallbladder adenocarcinomas, and two developed hepatocellular carcinomas. One monkey had an osteosarcoma and three were found at necropsy to have multiple primary tumors. Adenocarcinoma of the pancreas occurred in all monkeys with multiple primary tumors, coincident with urinary bladder carcinoma, adenocarcinoma of the bile ducts, or osteosarcoma. The tumors diagnosed in the 18 monkeys developed after an average latent period of approximately 10 years (range 5 to 12 years) and after an average cumulative AFB_1 dose of 820 mg (range 292 to 1103 mg).

Table 6. Summary of Control and AFB_1-Treated Monkeys[a]

Group	No. Alive	No. Dead			No. of Monkeys
		Without Tumor	With Tumor	(%)	
AFB_1[b]	6	21	18	(40.0)	45[c]
Control	129	83	7	(3.2)	219

[a]From 1961 to 1981.
[b]Monkeys received AFB_1 by po (0.1 to 0.8 mg/kg) and/or ip (0.125 to 0.25 mg/kg) routes; dosing began within one month of birth.
[c]25 Rh, 18 Cy, 2 Gr.

The carcinogenic potential of cycads (crude cycad meal, purified cycasin, and synthetic MAM-acetate) administered by po or ip routes has been under investigation for the past 12 years (7). Eighteen monkeys survived longer than two months after the first dose of cycasin (50 to 75 mg/kg) or MAM-acetate (1.5 to 3.0 mg/kg) given po, 5 days/week. Eleven of these animals have been necropsied and three had tumors (see Table 8). A group of 10 monkeys received MAM-acetate by weekly ip injections (3 to

Table 7. Tumors Induced by AFB_1

Tumor Type[a]	No. of Monkeys	Avg. Total Dose (mg)	Avg. Latent Period (mo)
Hemangiosarcoma: liver	7 (5 Rh, 2 Cy)	978	140
ACA: bile duct or gallbladder	5 (2 Rh, 3 Cy)	959	137
CA: hepatocellular	2 Rh	478	60
Osteosarcoma	1 Cy	412	115
CA: pancreas and urinary bladder	1 Gr	292	107
ACA: pancreas and bile duct; osteosarcoma	1 Rh	353	117
ACA: pancreas and bile duct	1 Rh	1103	142
Average		820	126

[a]ACA = adenocarcinoma; CA = carcinoma.

Table 8. Summary of Control and Cycad-Treated Monkeys[a]

Group	No. Alive	No. Dead: Without Tumor	No. Dead: With Tumor	(%)	No. of Monkeys[b]
Cycad[c]					
po	7	8	3 (2 Rh, 1 Cy)	(16.7)	18
ip	2	3	5 (1 Gr, 3 Rh, 1 Cy)	(50.0)	10
Control	129	83	7	(3.2)	219

[a]From 1961 to 1981.
[b]17 Rh, 9 Cy, 2 Gr.
[c]Monkeys received cycads (crude cycad meal, purified cycasin or synthetic methylazoxymethanol acetate) within three days of birth.

10 mg/kg). Eight of these animals have been necropsied, and five had tumors. In addition, one of the two surviving monkeys in the ip treatment group developed a hepatocellular carcinoma that was surgically resected approximately eight years ago. Table 9 lists the tumors diagnosed in monkeys receiving cycad products by ip or po routes. All five of the necropsied monkeys treated with MAM-acetate by the ip route developed hepatocellular carcinoma, and two of the five had multiple primary tumors. In one monkey, a liver hemangiosarcoma, renal carcinoma, and esophageal squamous cell carcinoma were diagnosed in addition to hepatocellular carcinoma; the other monkey developed a renal carcinoma, adenocarcinoma of the small intestine, and esophageal squamous cell carcinoma. The tumors developing in the monkeys treated by the ip route were diagnosed after an average latent period of 80 months (range 63 to 88 months) and after an average cumulative MAM-acetate dose of 6.65 g (range 3.88 to 9.66 g).

Three monkeys on po cycads have thus far developed tumors (see Table 9). One monkey developed multiple primary tumors (hepatocellular carcinoma, bile duct adenocarcinoma, and renal carcinoma), and the other two had hepatocellular carcinoma and pancreatic adenocarcinoma, respectively. These tumors developed after latent periods of 107, 69, and 179 months and after the ingestion of considerably more cycad material than the tumor-bearing monkeys in the ip treatment group had been given.

Sterigmatocystin has been under test for the past five years. It is being administered po, 1 day/week at 1 mg/kg (15 monkeys) and 2 mg/kg (15 monkeys)). Thus far, only one monkey in the 2-mg/kg group has been necropsied, and histopathologic examination of tissue from this animal revealed no evidence of tumor development, although severe toxic hepatitis with hyperplastic nodules was noted. The remaining 29 animals are in good health.

Our studies have failed to implicate either cyclamate or saccharin as carcinogens in nonhuman primates (8). Cyclamate has been administered po 5 days/week at 100 mg/kg (12 monkeys) and 500 mg/kg (11 monkeys) for the past 10 and one-half years. The 100-mg/kg dose corresponds, on an equivalent surface area basis, to a daily intake of 2.3 g/day/70 kg man. With an average of 384 mg cyclamate in a 10-oz diet drink, this dose is equivalent to drinking about 6 diet drinks/day; correspondingly, the 500-mg/kg dose is equivalent to ingesting approximately 30 diet drinks/day. Two monkeys at each of the dose levels have died, but no evidence of tumor was found at necropsy or after histological examination of their tissue.

Saccharin has been administered to 2 groups of 10 monkeys each at 25 mg/kg, 5 days/week. One group of monkeys has been receiving saccharin for an average of 122 months (range 120 to 124 months),

Table 9. Tumors Induced by Cycads

Species[a]	Sex	Total Dose (gm)	Latent Period (mo)	Tumor(s) Diagnosed[b]
IP Dosing[c]				
Gr	M	3.88	63	HCA
Cy	F	5.06	80	HCA Hemangiosarcoma: liver CA: kidney SCA: esophagus
Rh	F	6.27	87	HCA
Rh	F	8.38	88	HCA CA: kidney ACA: small intestine SCA: esophagus
Rh	M	9.66	86	HCA
PO Dosing				
Rh	M	13.71[d] 28.62[c]	107	HCA ACA: bile duct CA: kidney
Cy	M	43.0[e] 30.41[d] 18.10[c]	69	HCA
Rh	M	53.5[e] 74.99[d]	179	ACA: pancreas

[a]Gr = African green; Cy = cynomolgus; Rh = rhesus.
[b]HCA = hepatocellular carcinoma; CA = carcinoma; ACA = adenocarcinoma; SCA = squamous cell carcinoma.
[c]MAM-acetate.
[d]Cycasin.
[e]Cycad meal.

and the second group was entered on study approximately three years ago. The 25-mg/kg dose corresponds, on an equivalent surface area basis, to a daily intake of 5 cans of diet soda (120 mg saccharin/can) or 15 packages of Sweet'N Low® (40 mg saccharin/package)/day. Since the inception of the saccharin study, none of the animals has died, and there is no evidence of toxicity in any of the animals thus far.

Similarly, long-term DDT administration has not yielded tumors in our nonhuman primates. A total of 24 animals has received DDT by the po route (20 mg/kg), 5 days/week, in a study that has been under way for the past 134 months. Administration of DDT is discontinued after a dosing interval of 130 months is completed. Although five of the monkeys have died thus far, none was found to have developed tumor. The apparent cause of death in these animals was DDT-induced central nervous system toxicity, as all experienced severe tremors and convulsions immediately prior to death. The 19 surviving monkeys appear to be in good health.

The carcinogenic potential of arsenic has been under evaluation for approximately six years. A total of 20 monkeys has received sodium arsenate po (0.1 mg/kg) 5 days/week, and three monkeys in the group have died. The cause of death in the monkeys was unrelated to arsenic treatment, and the surviving monkeys are well and without signs of toxicity. Nine monkeys have received lung implants containing tobacco smoke condensate in a beeswax matrix; all are well and without evidence of toxicity approximately eight years after implantation of the material.

Model Rodent Carcinogens

With the exception of urethane (ethyl carbamate), no compound listed in Table 1 as a "model rodent carcinogen" has induced tumors in Old World monkeys. Starting within one month of birth, urethane (250 mg/kg) was given po, 5 days/week, to rhesus and cynomolgus monkeys. Urethane treatment was continued for a maxmimum of five years, during which time some animals also received whole body irradiation in 3 to 10 weekly courses (50 rads/course). Urethane treatment was discontinued 11 to 14 years ago, and since that time all monkeys have been observed. Of 40 monkeys receiving urethane, 30 survived 6 months or longer after the first dose, and 22 of them have been necropsied (see Table 10). Thus far, eight malignant tumors have been found in five (16.7%) of the 30 treated monkeys; two of the five monkeys with tumors had received irradiation in addition to urethane. One or more primary liver tumors (three cases of hemangiosarcomas and one case each of adenocarcinoma of intrahepatic bile ducts and hepatocellular carcinoma) were diagnosed in four monkeys in which additional tumors present included an ependymoblastoma and a pulmonary adenocarcinoma (see

Table 11). An adenocarcinoma of the jejunum was diagnosed in the fifth monkey. The animals with tumors had received an average cumulative urethane dose of 260 g (range 230 to 339 g); the latent period for tumor induction averaged 171 months (range 142 to 229 months).

Table 10. Summary of Control and Urethane-Treated Monkeys[a]

Group	No. Alive	No. Dead			No. of Monkeys
		Without Tumor	With Tumor	(%)	
Urethane[b]					
Irr[c]	3	10	2	(13.3)	15[d]
Not Irr	5	7	3	(20.0)	15[d]
Controls	129	83	7	(3.2)	219

[a]From 1961 to 1981.
[b]Monkeys received urethane (250 mg/kg) 5 days/week for a maximum of five years, and treatment was discontinued 11 to 14 years ago. Some monkeys also received 3 to 10 weekly courses of whole body irradiation (50 rads/course).
[c]Irr = irradiated.
[d]8 Rh, 7 Cy.

Earlier studies from this laboratory (9) demonstrated that single sc doses (10 mg) of 3-methylcholanthrene (3-MC) produced fibrosarcomas in the primitive prosimian, Tupaia glis (tree shrew). Of six treated animals, three died within four months, and the three survivors developed fibrosarcomas within 14 to 16 months. A similar study in Old World monkeys was initiated 20 years ago in which 3-MC was administered po (14 animals) and by sc injection (7 animals). Animals treated po received the compound at a dose of 20 to 120 mg/kg 5 to 7 days/week; sc injections were given at 10 to 40 mg/kg for a total of 1 to 12 doses. Dosing was discontinued after 5 years, and the animals have been observed for up to 15 years. Although 8 of the 14 animals in the po treatment group, and 5 of the 7 in the sc group have died, no tumors have been detected at necropsy or upon histopathologic examination of their tissue.

Table 11. Tumors in Monkeys Given Urethane

Species[a]	Sex	Irradiation (No. of Courses)	Total Dose (gm)	Latent Period (mo)	Tumor(s) Diagnosed[b]
Rh	M	10	230.1	155	ACA: bile duct ACA: lung
Rh	M	9	243.0	181	Hemangiosarcoma: liver Ependymoblastoma
Cy	M	None	244.1	142	Hemangiosarcoma: liver
Rh	F	None	245.8	149	HCA Hemangiosarcoma: liver
Rh	F	None	339.2	229	ACA: jejunum
Average			260.4	171	

[a]Rh = rhesus; Cy = cynomolgus.
[b]ACA = adenocarcinoma; HCA = hepatocellular carcinoma.

Similarly, our previous studies with benzo[a]pyrene (BP) showed that fibrosarcomas developed in the prosimian Galago crassicaudatus (bush baby) at the site of a single sc injection (9). However, no tumors have developed in a group of Old World monkeys that received multiple sc injections of BP (30 to 90 mg/kg); 9 out of a total of 17 animals have survived treatment with this compound and have been under observation for up to 18 years. Other model rodent carcinogens, that after prolonged administration to Old World monkeys have failed thus far to induce tumors, include dibenz[a,h]anthracene; 2-acetylaminofluorene; 2,7-bis(acetylamino)fluorene; 4-dimethylaminoazobenzene; and 3'-methyl-4-dimethylaminoazobenzene.

N-Nitroso Compounds

The nitroso compounds as a class appear to be potent carcinogens in nonhuman primates; all but one of these compounds (N-nitrosodimethylamine [DMNA]) have induced tumors in monkeys. A

summary of results obtained with nitrosomethylurea (MNU) is shown in Table 12. A group of 43 monkeys received MNU (10 to 20 mg/kg) by the po route in 5 doses/week. Eighteen monkeys have been necropsied, and nine were diagnosed with malignant tumors, yielding an overall tumor incidence of 21% (10). All tumors induced by MNU were squamous cell carcinomas of the esophagus and oropharynx (see Table 13), and developed after latent periods ranging between 57 and 133 months (average 93 months).

Table 12. Summary of Control and MNU-Treated Monkeys[a]

Group	No. Alive	No. Dead			No. of Monkeys
		Without Tumor	With Tumor	(%)	
MNU	25	9	9	(20.9)	43[c]
Controls[b]	129	83	7	(3.2)	219

[a]From 1961 to 1981.
[b]Includes non-treated animals, breeders, and vehicle-treated controls.
[c]18 Rh, 19 Cy, 2 (Rh x Cy)F_1, and 4 Gr.

A rough parallel has been observed between the cumulative MNU dose ingested and the degree of esophageal damage found at necropsy of the treated monkeys (see Table 14). No esophageal lesions were found in two monkeys that had ingested an average of 15.34 g MNU for a period averaging 26 months, whereas esophagitis accompanied by candidiasis and chronic inflammatory infiltrates were noted in the esophageal mucosa of two monkeys receiving an average of 19.90 g MNU for an average of 58 months. In five monkeys necropsied after having ingested an average of 47.40 g MNU for an average of 54 months, esophageal lesions were more severe and included esophageal epithelial atrophy, hyper- or dyskeratosis, and dysplasia. The nine monkeys with squamous cell carcinomas of the esophagus had received MNU for an average of 93 months (range 57 to 133 months); during this period they had ingested an average cumulative MNU dose of 120.0 g (range 53.2 to 180.6 g). Only one monkey receiving in excess of 53.2 g MNU was found at necropsy to be without a carcinoma; this monkey had ingested 201.41 g MNU over the course of 124 months, and histopathologic examination of sections of esophagus revealed chronic esophagitis and extensive esophageal dysplasia.

Table 13. Tumors in Monkeys Receiving MNU Orally

Monkey No.	Species[a]	Sex	Total Dose (gm)[b]	Latent Period (mo)[c]	Histological Diagnosis
617H	Cy	M	53.21	63	SCA[d]: pharynx and esophagus with invasion of mediastinal lymph nodes; squamous metaplasia: trachea
622H	Rh	F	65.74	57	SCA: soft palate, tongue, and esophagus with invasion into stomach
539G	Rh	F	108.14	72	SCA: mouth; SCA in situ: pharynx; squamous papillomas: tongue, pharynx, and esophagus; dyskeratosis: esophageal mucosa
540G	Rh	M	129.29	83	SCA: mouth and esophagus; squamous papilloma and hyperkeratosis: buccal mucosa; SCA: esophagus
627H	Rh	M	129.91	133	SCA: esophagus
538H	Rh	M	133.80	72	SCA: mouth, pharynx, and esophagus; multiple squamous papillomas: pharynx and esophagus
569G	Gr	M	137.21	124	SCA: mouth, pharynx, and esophagus
624H	Rh	F	142.32	129	SCA: gingiva and esophagus
579G	Rh	M	180.65	107	SCA: mouth and esophagus

[a]Cy = cynomolgus; Rh = rhesus; Gr = African green.
[b]MNU (10 to 20 mg/kg) was incorporated into a vitamin sandwich and given 5 times/week; dosing was initiated within one week of birth.
[c]Latent period is the time in months from the first dose of MNU until the clinical diagnosis of tumor.
[d]SCA = squamous cell carcinoma.

Table 14. Esophageal Lesions Found at Necropsy in Nonhuman Primates Given MNU

No. of Monkeys	Avg. Total Dose (gm)[a]	Months Dosed	Esophageal Pathology
2	15.34	26	None
2	19.90	58	Esophagitis, candidiasis
5	47.40	54	Hyper- or dyskeratosis
9	120.00	93	Squamous cell carcinoma

[a]MNU (10 to 20 mg/kg) was given po 5 days/week.

1-Nitroso-1-methyl-3-nitroguanidine (MNNG) is also being administered by the po route (0.4 mg/kg, 5 days/week). A group of 21 monkeys has received this compound for periods of up to eight years; thus far, two animals have died of causes unrelated to treatment with MNNG. The remaining 19 animals appear to be in good health and without signs of toxicity. However, three additional monkeys were given MNNG as a colon implant; two monkeys have been necropsied, and one monkey was diagnosed with a well-differentiated adenocarcinoma of the rectosigmoid junction. The latter monkey had received a total MNNG dose of 8.65 g, administered in gelatin cubes containing 5.3 to 42.7 mg MNNG, which were inserted into the colon 2 days/week.

Another nitroso compound, 1-nitrosopiperidine (PIP), is carcinogenic in nonhuman primates (see Table 15), regardless of whether it is administered by po or ip routes. Thus 11 out of 12 monkeys (92%) receiving PIP po (400 mg/kg), 5 days/week, developed tumors, as did 5 of the 11 (46%) animals given PIP by weekly ip (40 mg/kg) injection. All tumors induced by PIP were hepatocellular carcinomas. Although the latent period for tumor development was similar in the po and ip treatment groups (87 and 76 months, respectively), the average total PIP dose ingested by monkeys receiving po treatment (1742.5 g) was approximately 45-fold greater than that of monkeys in the ip treatment group (39.4 g).

Table 16 summarizes resulted obtained in two small groups of six monkeys each, treated with N-nitrosodipropylamine (DPNA) and DMNA. All monkeys given weekly ip injections of DPNA (40 mg/kg) have been necropsied and found to have hepatocellular carcinomas. In contrast, four of the six monkeys treated with bimonthly ip injections of DMNA (10 mg/kg) have been necropsied, and none have developed tumors. Although histopathologic examination of tissue

Table 15. Summary of Control and PIP-Treated Monkeys[a]

Group	No. Alive	No. Dead			No. of Monkeys[b]
		Without Tumor	With Tumor	(%)	
PIP					
PO[b]	0	1 (Rh)	11	(91.7)[d]	12
IP[c]	2 (1 Rh, 1 Cy)	4 (2 Gr, 2 Rh)	5	(4.5)[e]	11
Controls	129	83	7	(3.2)	219

[a]From 1961 to 1981. 23 Monkeys given PIP comprised 13 Rh, 7 Cy, and 3 Gr.
[b]PO doses of PIP (400 mg/kg) were given 5 days/week.
[c]IP doses (40 mg/kg) were given 1 day/week.
[d]6 Rh, 5 Cy.
[e]3 Rh, 1 Cy, 1 Gr.

Table 16. Summary of Control and DPNA- and DMNA-Treated Monkeys[a]

Group	No. Alive	No. Dead			No. of Monkeys
		Without Tumor	With Tumor	(%)	
DPNA[b]	0	0	6	(100.0)[c]	6
DMNA[d]	2	4	0	(0.0)	6
Controls	129	83	7	(3.2)	219

[a]From 1961 to 1981. 4 Rh and 2 Cy received DPNA; 2 Cy and 4 Rh received DMNA.
[b]DPNA (40 mg/kg) was given 1 day/week by ip injection.
[c]All six tumors were HCA and developed after an average total dose of 7.0 g and after an average latent period of 28 months.
[d]DMNA (10 mg/kg) was given 1 day/2 weeks by ip injection

from these animals revealed severe hepatotoxicity (e.g., toxic hepatitis, cirrhosis, and hyperplastic nodules), no tumors were detected.

Nitrosodiethylamine (DENA) is a potent and predictable hepatocarcinogen in Old World monkeys, inducing tumors when given either by the ip or po route of administration (see Table 17). Twenty-nine out of 41 monkeys receiving po doses of DENA (40 mg/kg) 5 days/week developed hepatocellular carcinomas. Bimonthly ip injections of DENA (40 mg/kg) induced hepatocellular carcinomas in 106 out of 131 monkeys. When administered po, DENA induced tumors earlier and at a lower cumulative dose in cynomolgus monkeys, as compared to African greens, with the group of rhesus monkeys intermediate between the two species. This apparent species difference was not observed, however, when DENA was given by the ip route.

Table 17. Hepatocellular Carcinoma Induced by DENA

Species[a]	No. of Animals	Avg. Total Dose (gm)[b]	Avg. Latent Period (mo)
PO			
Cy	14	18.0	26
Rh	12	25.4	49
Gr	3	55.1	105
IP			
Cy	38	1.56	17
Rh	51	1.95	17
Gr	12	1.32	16
Cy x rh	5	1.84	15

[a]Cy = cynomolgus; Rh = rhesus; Gr = African green.
[b]DENA (40 mg/kg) was given po 5 days/week and ip bimonthly.

Table 18 shows DENA as carcinogenic in the prosimian Galago crassicaudatus (bush baby). The tumors induced in this species are primarily muco-epidermoid carcinoma of the nasal cavity rather than the hepatocellular carcinomas found in Old World monkeys. All 10 bush babies given bimonthly ip doses of DENA (10 to 30 mg/kg) developed tumors of the nasal cavity. In two of these animals, carcinoma of the liver was also present, and in both cases metastases to the lungs or to intestinal lymph nodes were noted.

The average total dose of DENA inducing tumors in the bush babies (0.75 g) is considerably lower than that required to induce tumors in Old World monkeys and reflects the lower body weight of

Table 18. Muco-Epidermoid Carcinoma (M-E CA) of the Nasal Mucosa Induced in Bush Babies by DENA

Group	No. With M-E CA/Total	No. With Liver CA/Total	Avg. Total Dose (gm)[a]	Avg. Latent Period (mo)
DENA	10/10	2/10	0.75	23
Controls	0/13	0/13	-	-

[a]DENA was administered by bimonthly ip injections at 10 to 30 mg/kg.

the bush babies. The average latent period for tumor induction in this species (23 months) is comparable to that in Old World monkeys. No obvious reason exists for the marked difference in the site of DENA-induced tumors noted between Old World monkeys and the bush babies. Possibly, it is related to differences in the metabolism or distribution of DENA, and this possibility is currently being investigated in our laboratory.

At this time, we are employing DENA as a model hepatocarcinogen in Old World monkeys to examine the relationship between chronic (milligrams per kilograms) dose, cumulative dose, and latent period for tumor induction. To this end, groups of monkeys are being given bimonthly ip injections of DENA at doses of 0.1, 1, 5, 10, 20, and 40 mg/kg and are observed for the appearance of tumor. In the four groups of monkeys in which tumors have developed, we have found that the latent period increases as the milligram-per-kilogram dose decreases (see Table 19). The study is incomplete; consequently, a precise value for the minimum carcinogenic dose for DENA cannot be given. However, it appears that this value will be approximately 1.4 g. Table 19 also shows the total dose and observation period for DENA-treated monkeys that are currently alive and without evidence of tumor. The 10 monkeys in the 0.1-mg/kg group have been studied only eight months and have received a total DENA dose of approximately 1 mg. In the 1-mg/kg group, the 80-month observation period exceeds the latent period required by the 5-mg/kg dose, but the total DENA dose administered (0.768 g) is below the apparent carcinogenic dose (1.4 g). The four tumor-free monkeys in the 5-mg/kg group, however, have received an average total DENA dose that exceeds the apparent carcinogenic dose, and they have been observed for seven months longer than the average latent period for tumor induction at this dose level. The single tumor-free monkey at the 10-mg/kg dose level has received a total DENA dose (4.101 g) that is well in excess of the apparent carcinogenic dose. This monkey has been

observed for a period that is approximately twice the latent period for tumor induction by DENA at that dose.

DISCUSSION

The rodent has traditionally been the system in which bioassays for chemical carcinogenesis are carried out, and the results of such bioassays have provided much of the basis for estimating the risk that chemicals may pose to man. However, a number of problems exist when extrapolating rodent carcinogenesis data to man. These problems include the necessity of administering exceedingly high doses of the test chemical to compensate for the relatively short life span of mice and rats (11), the high incidence of spontaneous tumors in some rodent colonies in which bioassays are carried out (12), and the observation that rodents metabolize many chemicals via pathways that differ from those found in the human.

On the other hand, nonhuman primates are employed infrequently in long-term carcinogenesis studies, chiefly because of the expense of such an undertaking, the time period required to complete a lifetime study, the unavailability of sufficient numbers of animals, and the relatively large quantity of test chemical required. Nevertheless, a number of advantages exist when performing chemical carcinogenesis studies in nonhuman primates rather than in rodents. These advantages include the comparatively low incidence of spontaneous tumors arising in monkeys, the similarity of many of their metabolic pathways to those of the human (1,2), and the fact that their relatively long life span and large size enable the experimenter to administer chemicals by routes and by dosage schedules that closely parallel human exposure patterns. Thus, although nonhuman primates will never supplant rodents as the primary screening system for potentially carcinogenic chemicals, they can provide important information on the carcinogenic potential of chemicals in wide use, chemicals to which large numbers of humans are exposed, or chemicals for which data from rodents are ambiguous or conflicting.

The carcinogenic potential in nonhuman primates of a variety of chemicals, differing widely in chemical structures and properties, has been under investigation in this laboratory for the past 20 years. Prior to the inception of this study, nonhuman primates, unlike the rodent, were believed to be relatively resistant to tumor induction by chemicals. Therefore, an early objective of the present study was to evaluate the response of monkeys to chemicals known to be carcinogenic in rodents. A series of model rodent carcinogens, including 3-MC, BP, dibenz[a,h]anthracene, 2-acetylaminofluorene, and urethane, was accordingly tested. With the exception of urethane, none of these

Table 19. Induction of Hepatocellular Carcinoma (HCA) in Monkeys by Bimonthly IP Injections of DENA.

DENA Dose (mg/kg)	No. HCA/No. Treated	Monkeys with HCA		Monkeys Without HCA	
		Avg. Total Dose (gm)	Avg. Latent Period (mo)	Avg. Total Dose (gm)	Months Observed
0.1	0/10 (4 RH, 6 Cy)	-	-	0.001	8
1	0/10 (7 Rh, 1 Cy, 2 Gr)	-	-	0.768	80
5	6 (4 RH, 2 Cy)/10 (6 Rh, 3 Cy, 1 Gr)	3.128	65	2.872	72
10	9 (5 Rh, 4 Cy)/10 (5 Rh, 5 Cy)	1.780	34	4.101	63
20	11/11 (6 Rh, 5 Cy)	2.177	26	-	-
40	10/10 (5 Rh, 3 Cy, 1 F_1, 1 Gr)	1.430	17	-	-

model rodent carcinogens has proved to be carcinogenic in the Old and New World species of monkey employed in our studies, although both 3-MC and BP produced tumors in more primitive primates (bush babies and tree shrews), as they do in rodents (9). These results suggest that prosimian primates resemble the rodent more closely than they do macaques in their response to a carcinogenic stimulus.

Many of the chemicals classified in the present study as "food additives and environmental contaminants" are reported to be rodent carcinogens. Thus the artificial sweeteners cyclamate and saccharin as well as DDT have been (or may be) withdrawn from public use on the basis of their carcinogenicity in rodents. Nevertheless, neither the artificial sweeteners nor DDT have induced tumors in nonhuman primates despite extensive testing over prolonged time periods. The mold product sterigmatocystin is another example of a rodent carcinogen (13) that thus far is apparently devoid of carcinogenicity in nonhuman primates. However, both AFB_1 and cycad (particularly MAM-acetate) are potent carcinogens in rodents (14,15) and are carcinogenic in nonhuman primates as well. Although arsenic is suspected of being a human carcinogen (16), it has not induced tumors in rodents and has not as yet proved to be carcinogenic in nonhuman primates.

Many of the clinically useful antineoplastic and immunosuppressive agents are potent rodent carcinogens (17); however, they were tested in our monkey colony because of the increasing suspicion that they are etiologic agents in second malignant tumors arising in successfully treated cancer patients and in tumors arising in patients receiving chronic immunosuppressive therapy for collagen vascular disease or for renal homografts (18). Five chemotherapeutic agents have been evaluated for carcinogenic activity in our colony of nonhuman primates, but only one of these (procarbazine) has as yet demonstrated unequivocable carcinogenic properties. Adriamycin may be a leukemogen, but further studies are needed and are in progress. The other three drugs, azathioprine, melphalan, and cyclophosphamide, have probably not been tested long enough for their carcinogenic potential to become manifested.

With the exception of DMNA, all of the nitroso compounds induced tumors in nonhuman primates. MNU is a direct-acting carcinogen; the fact that po doses of this compound induced tumors of the oropharynx and esophagus is no surprise. However, another direct-acting carcinogen, MNNG, has not as yet induced tumors when administered po, although a colon implant containing this chemical produced an adenocarcinoma at the site of implantation. The remainder of the carcinogenic nitroso compounds are hepatocarcinogens in Old World monkeys, inducing a high yield of hepatocellular carcinoma in the treated animals. The chemical that we have acquired the most information about, DENA, is a predictable

and reproducible carcinogen, inducing tumors following either ip or po treatment. Extensive studies with this chemical have provided information pertaining to the relationship between chronic dose, cumulative dose, and latent period for tumor induction.

Our results indicate that the latent period for tumor induction increases as the chronic milligram-per-kilogram dose decreases and that the cumulative carcinogenic dose for DENA is approximately 1.4 g. Assuming an average body weight of 8 kg, the monkeys in the 1-mg/kg treatment group would receive approximately 208 mg of DENA/year. Taking the value 1.4 g as the carcinogenic dose for DENA, the latent period for tumor induction in these animals would be about six to seven years, a time interval well within the average life span (25 to 30 years) of Old World monkeys in captivity. However, in the 0.1-mg/kg group, the maximum total DENA dose that could be given over a 25- to 30-year period is approximately 0.52 to 0.62 g, a cumulative dose that is considerably lower than the apparent carcinogenic dose for DENA.

Some insight into low-dose extrapolation of carcinogenic risk may be gained by considering a semilog plot of the milligram-per-kilogram dose of DENA against the latent period for tumor induction (Figure 1; Table 19). The 40, 20, and 10 mg/kg points fall on a straight line that intersects the ordinate at 88 months. This point on the ordinate corresponds to a DENA dose of 0.1 mg/kg. Thus, animals in the 0.1-mg/kg group should develop tumors after a latent period of 88 months if the relationship between milligram-per-kilogram dose and latent period is strictly linear; however, the animals receiving this dose have only been under observation for eight months (Table 19). The line passes through the ordinate at 60 months for the 1-mg/kg group, although this group remains tumor free after 80 months of observation. The tumors developing in the six animals receiving the 5-mg/kg dose required a latent period of 65 months, a figure that shows a marked deviation from the value (42 months) expected if the relationship between dose and latent period is indeed linear.

ACKNOWLEDGMENTS

This work is being supported in part by National Cancer Institute contract NO1-CM-33708 with Hazleton Laboratories America, Inc., Vienna, Virginia.

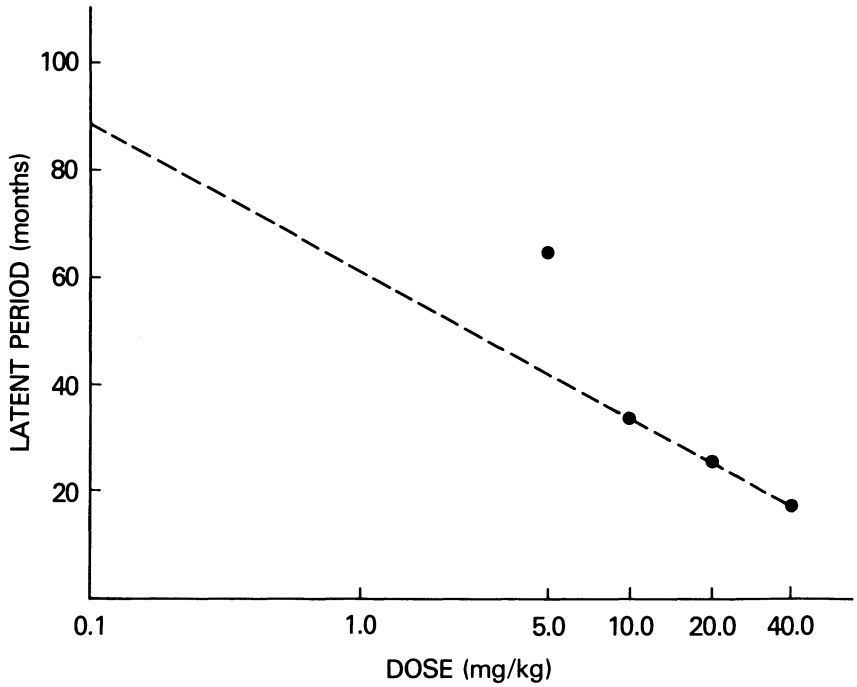

Figure 1. Relationship between chronic (milligram-per-kilogram) DENA dose and latent period for tumor induction in Old World monkeys receiving bimonthly ip injections of DENA at 0.1, 1, 5, 10, 20, and 40 mg/kg.

REFERENCES

1. Smith, R.L., and R.T. Williams. 1974. Comparative metabolism of drugs in man and monkeys. J. Med. Primatol. 3:138-152.

2. Smith, R.L., and J. Caldwell. 1977. Drug metabolism in non-human primates. In: Drug metabolism - from microbe to man. D.V. Parke, and R.L. Smith, eds. Taylor and Francis Ltd.: London. pp. 331-356.

3. Adamson, R.H. 1972. Long-term administration of carcinogenic agents to primates. In: Medical Primatology 1972, Part III. E.I. Goldsmith, and J. Moor-Jankowski, eds. S. Karger: Basel, Switzerland. pp. 216-225.

4. Sieber, S.M., P. Correa, D.W. Dalgard, and R.H. Adamson. 1978. Carcinogenic and other adverse effects of procarbazine in nonhuman primates. Cancer Res. 38:2125-2134.

5. Sieber, S.M., P. Correa, D.M. Young, D.W. Dalgard, and R.H. Adamson. 1980. Cardiotoxic and possible leukemogenic effects of adriamycin in nonhuman primates. Pharmacol. 20:9-14.

6. Sieber, S.M., P. Correa, D.W. Dalgard, and R.H. Adamson. 1979. Induction of osteogenic sarcomas and tumors of the hepatobiliary system in nonhuman primates with aflatoxin B_1. Cancer Res. 39:4545-4554.

7. Sieber, S.M., P. Correa, D.W. Dalgard, K.R. McIntire, and R.H. Adamson. 1980. Carcinogenicity and hepatoxicity of cycasin and its aglycone, methylazoxymethanol-acetate in nonhuman primates. J. Natl. Cancer Inst. 65:177-189.

8. Sieber, S.M., and R.H. Adamson. 1978. Long-term studies on the potential carcinogenicity of artificial sweeteners in nonhuman primates. In: Health and Sugar Substitutes. B. Guggenheim, ed. S. Karger: Basel, Switzerland. pp. 266-271.

9. Adamson, R.H., R.W. Cooper, and R.W. O'Gara. 1970. Carcinogen-induced tumors in primitive primates. J. Natl. Cancer Inst. 45:555-559.

10. Adamson, R.H., F.J. Krowlikowski, P. Correa, S.M. Sieber, and D.W. Dalgard. 1977. Carcinogenicity of 1-methyl-1-nitrosourea (MNU) in nonhuman primates. J. Natl. Cancer Inst. 59:415-422.

11. Sontag, J.M. 1977. Aspects in carcinogen bioassay. In: Origins of Human Cancer, Book C. H.H. Hiatt, J.D. Watson, and J.A. Winsten, eds. Cold Spring Harbor Laboratory: New York. pp. 1327-1338.

12. Weisburger, J.H., D.P. Griswold, J.D. Prejean, A.E. Casey, H.B. Wood, and E.K. Weisburger. 1975. The carcinogenic properties of some of the principle drugs used in clinical cancer chemotherapy. Recent Results Cancer Res. 52:1-17.

13. Purchase, I.F.H., and J.J. Van der Watt. 1970. Carcinogenicity of sterigmatocystin. Food Cosmet. Toxicol. 8:289-293.

14. Butler, W.H. 1965. Liver injury and aflatoxin. In: Mycotoxins in Foodstuffs. G.N. Wogan, ed. Massachusetts Institute of Technology Press: Cambridge, MA. pp. 175-186.

15. Laqueur, G.L. 1964. Carcinogenic effects of cycad meal and cycasin (methylazoxymethanol glycoside) in rats and effects of cycasin in germfree rats. Fed. Proc. 23:1386-1388.

16. Hernberg, S. 1977. Incidence of cancer in population with exceptional exposure to metals. In: Origins of Human Cancer, Book A. H.H. Hiatt, J.D. Watson, and J.A. Winsten, eds. Cold Spring Harbor Laboratory: New York. pp. 147-157.

17. Sieber, S.M., and R.H. Adamson. 1975. The clastogenic, mutagenic, teratogenic, and carcinogenic effects of various antineoplastic agents. In: Pharmacological Basis of Cancer Chemotherapy. Williams and Wilkins Co.: Baltimore, MD. pp. 401-468.

18. Sieber, S.M., and R.H. Adamson. 1975. Toxicity of antineoplastic agents in man: Chromosomal aberrations, antifertility effects, congenital malformations, and carcinogenic potential. Adv. Cancer Res. 22:57-155.